報告した論文もある[10]．HCVの発見後は献血・輸血時のHCVスクリーニング法が確立したために，輸血後C型肝炎の発症は極めて稀となった．日本の報告では，Nakashimaら[6]はHCVの感染経路として輸血の次に性行為をあげている．

本報告では，患者本人とフィアンセのHCV遺伝子型が同じ2型であった．両者の血清を検索しHCV RNAの塩基配列が高率な相同性を示し分子系統樹で同じclusterを形成したことと，病歴聴取より感染危険因子である歯ブラシ・カミソリなどの日用品や注射針を共有したことが無いことを確認できたことから性行為以外の共通行為は考えにくいためフィアンセ間の性感染である可能性が高いと推測した．2005年，Nakayamaらも同様の方法でC型急性肝炎を発症した配偶者の報告を行っている[11]．本患者はフィアンセと07年8月頃から付き合い始め，急性肝炎発生3カ月前頃から性交渉があったと話している．2007年11月頃ほぼ同時に急性肝炎で発症しているが，確定は出来ないがフィアンセから本患者にHCV感染が起こった可能性が考えられる．

一方，C型急性肝炎は約70％の確率で慢性化することが知られている[8]．いったん慢性化すると自然治癒は稀で徐々に線維化が進行し肝硬変から肝細胞癌の合併が高頻度となる．HCV消失が認められる例が約30％存在するが，消失する場合は発生3カ月以内に認められ，それ以降の消失は稀である．発生3カ月を過ぎても，HCV陽性の症例は慢性化している可能性が高く，インターフェロン治療が勧められるとしている．急性肝炎発生早期でのインターフェロン治療は慢性肝炎に比し高いウイルス駆除が得られることが知られている[12][13]．しかし，報告症例数が少なく治療開始時期や治療法の明確な基準は示されていない．Kamalら[14]はC型急性肝炎に対するペグインターフェロンα2b治療開始時期の検討をしている．このなかで1型は8週以内に投与開始した成績が最も良好であったが，2型は治療開始時期によらず90％以上のウイルス駆除が得られたとしている．治療にリバビリンの併用は必須ではなく，ペグインターフェロン単独で十分と結論している[15]が，投与期間について1型は24週投与が必要だが2型は8から12週投与で良いと報告している[16]．今回の症例は2型であり，ペグインターフェロンα2a単独投与を12週行ってHCV RNAは陰性化した．2型はインターフェロンの反応が良好であるので，2型のC型急性肝炎の治療を何時行うべきであるかは異論があるが，女性は年齢が進むほど治癒率が低下すると言われている[17]．本症例では早期のインターフェロンが望ましいと考え，発生5カ月後の肝生検で慢性化を確認の上でペグインターフェロンα2a投与を行いHCVの消失を認めた．

結　語

分子系統樹解析からフィアンセから感染したと推測されたC型急性肝炎の女性症例を経験した．急性肝炎後に慢性化したため早期のペグインターフェロン治療を行いC型肝炎は治癒したと思われた．若い世代を中心に麻薬や刺青によるC型肝炎感染が散見され，性交渉によってHCVがさらに拡大しているとも言われている．日本のHCV感染者は高齢化し治療に難渋している．治療反応性の良い若い世代に対する積極的HCV対策も今後は必要である．

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A case of acute hepatitis C, most likely by interlovers sexual transmission, cured by peginterferon α2a therapy

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A 29-year-old woman with acute hepatitis C visited our hospital at November 21, 2007. ALT did not become normal. HCV serotype revealed Group 2 and HCV RNA was 3.0 Log IU/ml (Real time method) at March 21, 2008. Liver Biopsy showed chronic hepatitis F1A1 at March 21, so she was treated with pegylated interferon α 2a for 12 weeks and attained sustained virological response. On the other hands, her fiancé treated for acute hepatitis C at another hospital revealed HCV Group 2 and HCV RNA was 430 KIU/ml (High ranged method). The HCV isolates from the patients and her fiancé shared 100% identity in the 338 nucleotide sequences of the NS5B region. Phylogenitic analysis of the 338 sequences revealed that the two isolates segregated into a cluster. Thus the patient has acquired HCV infection from her fiancé, most likely by sexual transmission.

Key words: acute hepatitis C　pegylated interferon α 2a　sexual transmission　phylogenitic analysis

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The level of fasting serum insulin, but not adiponectin, is associated with the prognosis of early stage hepatocellular carcinoma

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Abstract. Impaired glucose tolerance influences the prognosis of hepatocellular carcinoma (HCC), but this mechanism is still not fully understood. We investigated the impact of the fasting serum levels of insulin and adiponectin on the prognosis of HCC and its recurrence. One hundred and forty patients with newly diagnosed HCC were enrolled in the prognosis study. Their fasting serum levels of insulin and adiponectin were determined. Of 140 patients, 59 patients who underwent curative treatment were subjected to analysis of the recurrence-free survival. The 140 patients were divided into two groups by the 50th percentile value of insulin (7.73 μIU/ml) or total adiponectin (6.95 μg/ml). Kaplan-Meier analysis indicated that high insulin group (>7.73 μIU/ml) exhibited a significantly poorer prognosis than low insulin group (<7.73 μIU/ml) in early stage HCC (P=0.018). In contrast, the level of total adiponectin had no impact on the prognosis of HCC. Multivariate analysis indicated that fasting hyperinsulinemia was an independent risk factor for a poorer prognosis in early stage HCC (P=0.044). Likewise, Kaplan-Meier analysis indicated that the recurrence-free survival of high insulin group was significantly lower than that of low insulin group (P=0.017). The level of total adiponectin had no impact on the recurrence-free survival of HCC. Multivariate analysis indicated that fasting hyperinsulinemia was an independent risk factor for the lower recurrence-free survival of HCC (P=0.049). In conclusion, our study suggests that the fasting insulin level affects the clinical course of early stage HCC.

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Key words: hepatocellular carcinoma, insulin, adiponectin, prognosis, recurrence

Introduction

Hepatocellular carcinoma (HCC) is the fifth most frequent malignant neoplasm in the world (1). Extensive evidence of the rising incidence of HCC has been reported in the United States, Japan, and several other countries (2,3). In addition to chronic infection by the hepatitis C virus (HCV), diabetes mellitus (DM) is thought to be a rising risk factor of note, because it is associated with nonalcoholic fatty liver disease (NAFLD) including its severe form, nonalcoholic steatohepatitis (NASH) (4). NASH is a chronic necroinflammatory condition that can lead to liver fibrosis, cirrhosis, and subsequently to HCC (5,6). In earlier studies it was suggested that there was no evidence linking DM to HCC, whereas more recent studies have indicated the association between DM and HCC (6-9). Moreover, several studies have reported that the coexistence of DM in chronic liver disease caused by chronic infection of HBV and HCV is closely related to the risk of not only the development of HCC, but also a poor prognosis (10,11). However, it is not clear why coexisting DM influences the development and progression of HCC in chronic liver disease.

The abnormality of the glucose metabolism found in chronic liver disease is due to the existence of a decline of insulin degradation, and peripheral insulin resistance (12). We have also reported that the developing of liver fibrosis is closely associated with insulin resistance in HCV infected patients (13). Taken together, it is likely that insulin resistance in chronic liver disease triggers hyperinsulinemia, and it may modulate the biological characteristics of HCC cells. It has been reported that insulin displays growth promoting and anti-apoptotic effects on human hepatoma cells *in vitro* and in animal models (10,14,15). Moreover, Saito *et al* have

previously reported that postprandial hyperinsulinemia is associated with the accelerated growth of HCC (16).

Recently, adiponectin, a physiologically active polypeptide secreted exclusively by adipose tissue, has been the focus of research interest as a factor involved in glucose metabolism. This hormone has a potent insulin-sensitizing effect (17-19), and a low level of circulating adiponectin is found in several types of metabolic syndrome including insulin resistance and type 2 diabetes (20). Several studies have reported the association between the values of circulating adiponectin and liver disease. Xu *et al* have reported that adiponectin administration alleviates hepatomegaly and steatosis and also significantly attenuates the inflammation and the elevated levels of serum alanine aminotransferase in alcoholic and nonalcoholic fatty liver murine models (21). In humans, the level of circulating adiponectin has been found to increase in patients with advanced cirrhosis (22-24). In addition, Hui *et al* reported that the level of serum adiponectin increases in advancing liver fibrosis and declines with a reduction in fibrosis in chronic hepatitis B (25).

The aim of the present study was to clarify whether the levels of fasting serum insulin and adiponectin are relevant to the prognosis of HCC in patients newly diagnosed to have HCC and the recurrence of HCC in those who underwent curative therapy.

Patients and methods

Patients. A total of 140 patients, who were newly diagnosed to have HCC between January 1995 and December 2004 at the First Department of Internal Medicine in Nagasaki University Hospital and fulfilled the criteria specified below, were enrolled in the current cohort. The inclusion criteria were: 1) not diagnosed as having DM on admission, in other words, no dietary intervention and no regular use of medication to affect insulin sensitivity or insulin secretion, 2) on admission a fasting serum sample was drawn and stored, and 3) no life-threatening illness other than liver disease.

The diagnosis of HCC was based on the typical findings detected by ultrasonography (US), dynamic computerized tomography (CT), magnetic resonance imaging (MRI), abdominal angiography, and/or histological manifestation of liver tumor. Underlying liver diseases, such as chronic hepatitis (CH) and liver cirrhosis (LC) were diagnosed by liver histologic examination following a liver biopsy and/or by the findings of US, dynamic CT, and MRI. LC was graded according to the Child-Pugh classification (26). The body mass index (BMI) was calculated as body weight in kilograms divided by the square of the height in meters (kg/m^2). The alcohol intake was assessed by interview and recorded in grams per day. The patients were divided into two groups according to the mean alcohol consumption per day; not excessive drinkers (<80 g/day) and excessive drinkers (≥80 g/day). Fasting blood samples were obtained in the early morning for an analysis of biochemical and hematological data or fasting blood glucose, and serum samples were stored at -80°C until further assay. Hepatitis B surface antigen (HBsAg) and hepatitis C virus antibody (HCVAb) were tested by commercial immunoassays (Fuji Rebio, Tokyo). The serum AFP level was measured by enzyme immunoassay (AxSYMAFP, Abbott Japan, Tokyo).

Table I. Clinical and laboratory characteristics of the study subjects.

Variable	Number or mean (SD)
Patients	140
Onset age, y.o.	65.1 (9.5)
Gender	
Male	110
Female	30
BMI, kg/m^2	23.1 (3.1)
Alcohol intake	
<80 g/day	108
≥80 g/day	32
Etiology	
HBsAg(+)	29
HCVAb(+)	99
non-B, non-C	12
Underling liver diseases and Child-Pugh grade	
CH	30
LC grade A	72
LC grade B	31
LC grade C	7
Total bilirubin, mg/dl	1.4 (2.2)
Ferritin, ng/ml	304.2 (345.2)
Serum iron, μg/ml	151 (75)
Fasting insulin, μIU/ml	10.0 (9.6)
Fasting blood glucose, mg/dl (n=62)	102.7 (51.5)
HOMA-R, % (n=28)	3.0 (2.6)
Total adiponectin, μg/m	8.1 (4.8)
HMW, μg/ml	3.9 (3.0)
MMW, μg/ml	1.8 (1.2)
LMW, μg/ml	2.4 (1.3

Measurement of insulin and adiponectin. Fasting serum samples stored at -80°C were used for the assay. Fasting serum insulin was measured by enzyme immunoassay (Fuji Rebio). Fasting total adiponectin was measured by an enzyme immunoassay (Daiichi Pure Chemicals Co., Ltd., Tokyo). Serum adiponectin exists in a complex form and is classified according to its molecular weight. Therefore, high molecular weight (HMW), middle molecular weight (MMW), and low molecular weight (LMW) adiponectins were also measured separately by enzyme immunoassay (Daiichi Pure Chemicals Co.).

HCC assessment. The size and number of HCCs were confirmed by US, CT, MRI, or angiography. We used the

Table II. HCC characteristics of the study subjects.

Variable	Number or mean (SD)
Tumor size, cm	3.4 (2.8)
<2 cm	57
2 - <5 cm	62
≥5 cm	21
Number of tumor lesions	
1	75
2	29
3 - and diffuse	36
TNM stage	
I	39
II	53
III	37
IV	11
AFP, ng/ml	7792.9 (62230.8)
Therapy	
Surgical resection	7
PEIT and/or RFA	53
TACE or TAI	73
Others	7

tumor-node-metastasis (TNM) classification system of the Liver Cancer Study Group (LCSG) of Japan in 2000 (27). The T category is determined by the 3 factors of number, size, and vascular or bile duct invasion. The N category is the presence of lymph node metastasis, and the M category is the presence of distant metastasis. TNM staging has four stages according to the T, N, and M categories. The therapy for HCC was divided into four groups; the surgical resection group, percutaneous ethanol injection therapy (PEIT) and/or radiofrequency ablation (RFA) group, transcatheter arterial chemoembolization (TACE) or transarterial infusion (TAI) group, and other therapy or palliative therapy group. In this study, the curative therapy was defined as a condition characterized by the no findings of recurrence over six months after the initial therapy for HCC, including surgical resection, PEIT, RFA, and TACE or TAI.

Statistical analysis. The data were analyzed by the Mann-Whitney test for continuous ordinal data, χ^2 test with Yates' correction and Fischer exact test for the association between 2 qualitative variables, and Kaplan-Meier survival analysis. Parametric comparisons were assessed by analyses of variance. The significance of individual differences was evaluated by use of Scheffe's test. $P<0.05$ was considered to be statistically significant.

Results

Clinical features of studied patients. A total of 140 HCC patients were enrolled in this study. Patient characteristics are presented in Table I. There were 110 men (78.6%) and 30 women (21.4%). The mean age was 65.1 years. The mean BMI was 23.1 kg/m^2. Excessive drinkers comprised 22.9% (32 of 140) and not excessive was 77.1% (108 of 140). Patients who were HBsAg positive was 20.7% (29 of 140), whereas 70.7% (99 of 140) were HCVAb positive, and 8.6% (12 of 140) were negative for both (non-B, non-C). Chronic hepatitis (CH) was 21.4% (30 of 140). Liver cirrhosis (LC) was 78.6% (110 of 140). The Child-Pugh grade of LC patients was: grade A: 51.4% (72 of 140), grade B: 22.1% (31 of 140), and grade C: 5.0% (7 of 140). The mean level of total bilirubin was 1.4 mg/dl. The mean levels of ferritin and serum iron were 304.2 ng/ml and 151 μg/ml, respectively. The mean level of fasting insulin was 10.0 μIU/ml. The mean level of fasting blood sugar in 62 patients measured during the hospital stay was 102.7 mg/dl. In a similar fashion, the level of HOMA-R in the 28 patients calculated was 3.0%. The mean values of total, HMW, MMW, and LMW adiponectins were 8.1, 3.9, 1.8 and 2.4 μg/ml, respectively.

The characteristics of newly diagnosed HCC on admission are presented in Table II. The mean size of HCC was 3.4 cm and its distribution was: <2 cm: 57 (40.7%), ≥2 cm and <5 cm: 62 (44.3%), and ≥5 cm: 21 (15.0%). The number of HCCs in the subjects was: 1: 75 (53.6%), 2: 29 (20.7%), and 3 or more and diffuse: 36 (25.7%). The TNM stage of the HCC was: stage I: 39 (27.9%), stage II: 53 (37.9%), stage III: 37 (26.4%), and stage IV: 11 (7.9%). The mean level of AFP was 7792.9 ng/ml. Of the studied patients, 5.0% (7 of 140) underwent surgical resection, 37.9% (53 of 140) underwent PEIT and/or RFA, 52.1% (73 of 140) underwent TACE or TAI, and 5.0% (7 of 140) received other therapy or palliative care only.

The values of fasting insulin and adiponectin in subjects. We evaluated the values of fasting insulin and total adiponectin in underlying liver diseases or in the TNM stage of HCC. The mean values of insulin of CH, LC with Child-Pugh grade A, B, and C were 6.9, 10.3, 11.9, and 12.7 μIU/ml, respectively (Fig. 1A). The mean value of insulin seemed to increase in LC (Child-Pugh grade A, B, C) compared to CH, but no significant differences were observed between them. The mean values of insulin of TNM stage I, II, III, and IV were 8.1, 11.1, 11.5, and 7.1 μIU/ml, respectively. No significant differences were observed in these groups.

The mean values of total adiponectin of CH, LC with Child-Pugh grade A, B, and C were 6.4, 7.7, 9.5, and 13.4 μg/ml, respectively (Fig. 1B). In parallel with the decline of liver function, the mean value of total adiponectin increased obviously, and the mean value of total adiponectin of LC with Child-Pugh grade C showed a significantly higher level than that of CH and LC with Child-Pugh grade A. In contrast, the mean values of total adiponectin of TNM stage I, II, III, and IV were 8.5, 8.0, 8.3, and 6.6 μg/ml, and there were no significant differences.

Association of fasting insulin level with prognosis of HCC. To evaluate the association of fasting insulin level with the prognosis of HCC, the 140 patients were divided into two groups in terms of the 50th percentile of the value of insulin (7.73 μIU/ml). The mean level of insulin in the low insulin

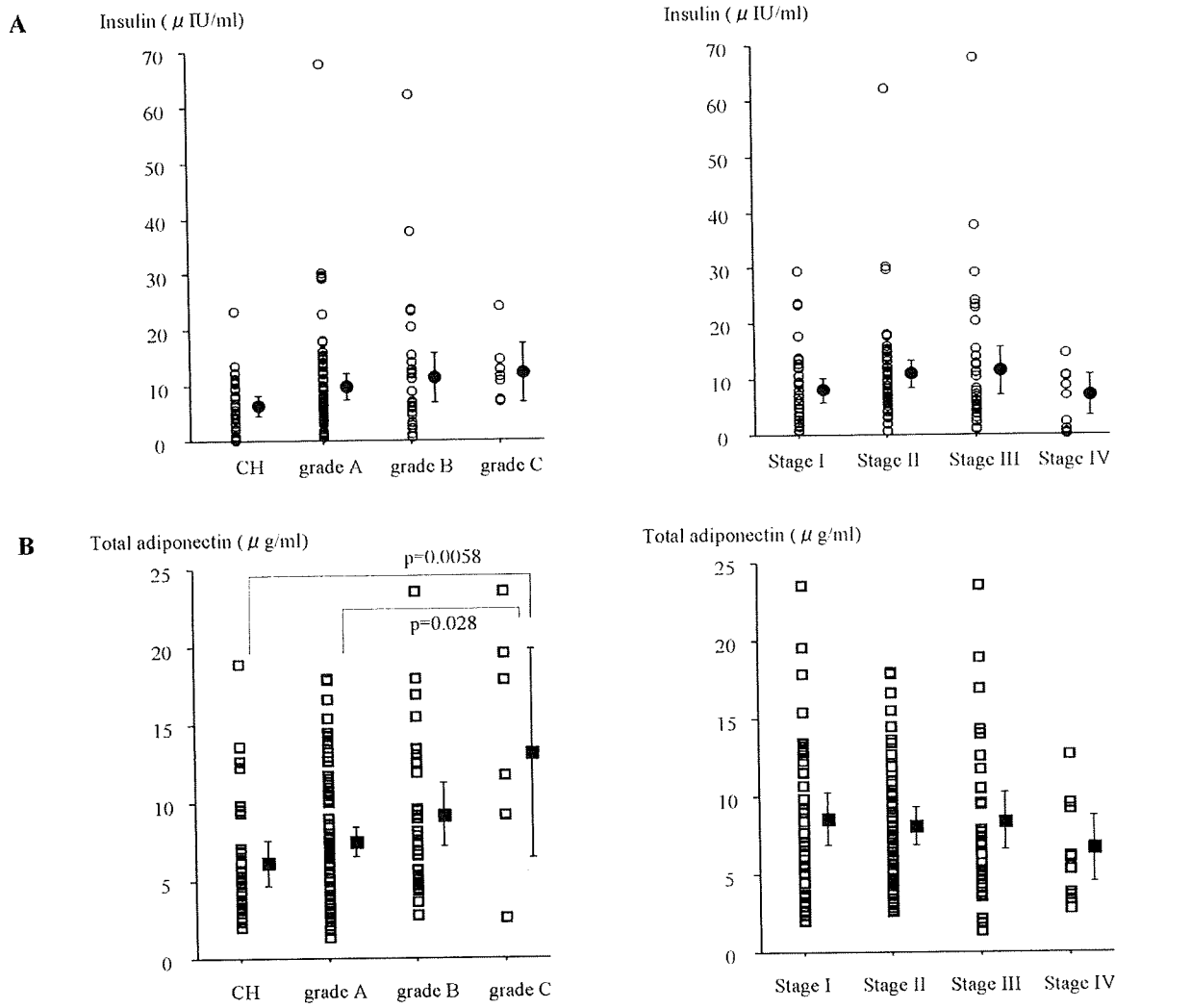

Figure 1. The mean ± SD values of fasting insulin in each underlying liver disease (CH and Child-Pugh grade) (A) and TNM stage (B), the mean ± SD values of total adiponectin in each underlying liver disease (CH and Child-Pugh grade) (C) and TNM stage (D).

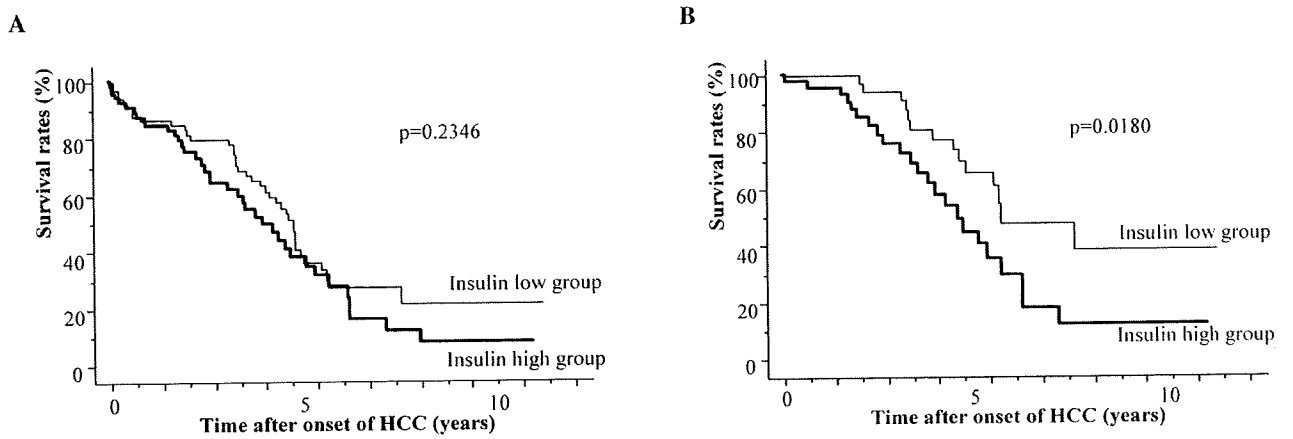

Figure 2. Kaplan-Meier curves for survival between low insulin group (thin line) and high insulin group (heavy line) in all stages of HCC patients (n=140) (A) and in TNM stage I + II HCC patients (n=92) (B).

group (<7.73 μIU/ml, n=70) was 4.3 μIU/ml. On the other hand, that in the high insulin group (>7.73 μIU/ml, n=70) was 15.8 μIU/ml. Table III shows the comparison of patient characteristics between the low and high insulin groups. The BMI in the high insulin group was significantly higher than it was in the low insulin group. The HOMA-R level was

Table III. Comparison of characteristics between low insulin group and high insulin group.

Variable	Number or mean (SD)		P
	Low insulin group (n=70)	High insulin group (n=70)	
Onset age, y.o.	64.7 (9.5)	65.5 (9.5)	0.620
Gender			0.999
Male	55	55	
Female	15	15	
BMI, kg/m^2	22.5 (3.0)	23.7 (3.0)	0.019
Alcohol intake			0.687
<80 g/day	53	55	
≥80 g/day	17	15	
Etiology			0.818
HBsAg(+)	16	13	
HCVAb(+)	48	51	
Non-B, non-C	6	6	
Underlying liver diseases and Child-Pugh grade			0.539
CH	17	13	
LC grade A	37	35	
LC grade B	14	17	
LC grade C	2	5	
Total bilirubin, ng/ml	1.5 (2.9)	1.3 (1.3)	0.634
Ferritin, ng/ml	305.2 (351.7)	303.2 (341.2)	0.974
Serum iron, μg/ml	155 (77)	148 (74)	0.571
Fasting insulin, μIU/ml	4.3 (2.2)	15.8 (10.6)	<0.001
Fasting blood glucose, mg/dl	94.0 (16.6) (n=31)	111.4 (70.5) (n=31)	0.185
HOMA-R, %	0.9 (0.6) (n=13)	4.8 (2.4) (n=15)	<0.001
Total adiponectin, μg/ml	7.9 (4.3)	8.3 (5.3)	0.611
HMW, μg/ml	3.7 (2.7)	4.1 (3.3)	0.473
MMW, μg/ml	1.9 (1.2)	1.8 (1.1)	0.570
LMW, μg/ml	2.3 (1.1)	2.5 (1.4)	0.600

calculated in 28 patients, and the level of HOMA-R in the high insulin group was significantly higher than that in the low insulin group. Table IV shows the comparison of characteristics of HCC between the two groups. Patients with more than three HCC lesions and diffuse HCC were more prevalent in the low insulin group than in the high insulin group. The other characteristics of HCC did not differ substantially between the two groups.

Fig. 2A indicates the cumulative survival rates of all stage HCC patients between the low insulin group (70 of 140) and the high insulin group (70 of 140). There was no significant difference between the two groups (P=0.235). Next, to evaluate the relationship of the fasting insulin level with the prognosis of early stage HCC patients, we analyzed the cumulative survival rates in HCC patients with TNM stage I and II disease (n=92). As shown in Fig. 2B, the high insulin group (49 of 92) exhibited a poor prognosis with a significant difference in comparison to the low insulin group (43 of 92) (P=0.018).

Association of fasting total adiponectin level with prognosis of HCC. Similarly, we evaluated the association of the total adiponectin level with the prognosis of HCC. One hundred and forty patients were divided into 2 groups in terms of the 50th percentile of the value of total adiponectin (6.95 μIU/ml). The mean level of total adiponectin in the low adiponectin group (<6.95 μIU/ml, n=70) was 4.5 μg/ml. That in the high adiponectin group (≥6.95 μIU/ml, n=70) was 11.8 μg/ml. We estimated the cumulative survival rates of all stages of HCC and early stage HCC between the low adiponectin group and the high adiponectin group. Fig. 3A and B show each result. No significant differences were found in all stages of HCC, or in the early stage of HCC (all stage HCC: low group vs. high group; P=0.886, early stage HCC: low group vs. high group; P=0.804).

Univariate and multivariate analyses of the factors associated with HCC prognosis. Univariate and multivariate analyses

Table IV. Comparison of HCC characteristics between low insulin group and high insulin group.

Variable	Number or mean (SD)		P
	Low insulin group (n=70)	High insulin group (n=70)	
Tumor size, cm	3.6 (3.2)	3.2 (2.5)	0.479
Number of tumor lesion			0.032
1	36	39	
2	10	19	
3 - and diffuse	24	12	
TNM stage			0.300
I	22	17	
II	21	32	
III	21	16	
IV	6	5	
AFP, ng/ml	5136.8 (30566.9)	10487.6 (83045.4)	0.614
Therapy			0.492
Surgical resection	5	2	
PEIT and/or RFA	27	26	
TACE or TAI	35	38	
Others	3	4	

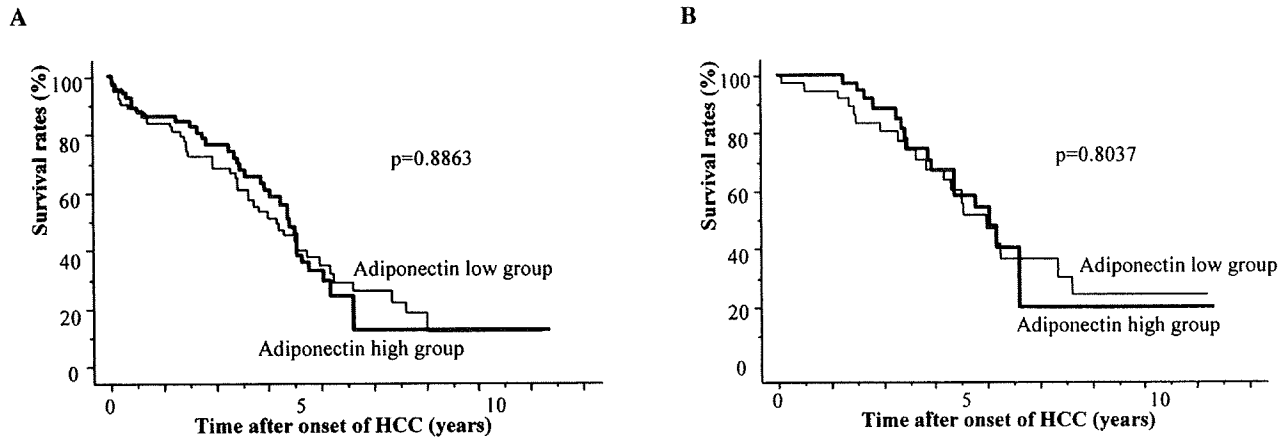

Figure 3. Kaplan-Meier curves for survival between low adiponectin group (thin line) and high adiponectin group (heavy line) in all stages of HCC patients (n=140) (A) and in TNM stage I + II HCC patients (n=92) (B).

using the Cox proportional hazards model in 140 patients diagnosed with HCC were performed to identify the relevant independent prognostic factors in all stages of HCC. In a univariate analysis, the following three factors significantly influenced the prognosis: alcohol intake (excessive drinker, RR 2.033, 95% CI 1.206-3.425, P=0.008), Child-Pugh grade (grade C, RR 9.906, 95% CI 3.547-27.666, P<0.001), and therapy for HCC (TACE or TAI, RR 1.856, 95% CI 1.143-3.015, P=0.012). However, a multivariate analysis revealed that only two factors influenced the HCC prognosis significantly: Child-Pugh grade (grade C, RR 9.807, 95% CI 2.710-30.471, P<0.001) and therapy for HCC (TACE or TAI, RR 1.803, 95% CI 1.104-2.943, P=0.018).

Next, univariate and multivariate analyses in 92 patients diagnosed with HCC, all TNM stage I or II, were performed to identify the independent prognostic factors of early stage HCC. In the univariate analysis, the following three factors significantly influenced prognosis: alcohol intake (excessive drinker, RR 2.488, 95% CI 1.160-5.319, P=0.019), Child-Pugh grade (grade B, RR 4.582, 95% CI 1.370-15.323, P=0.014, grade C, RR 41.104, 95% CI 6.403-263.831, P<0.001), and the value of insulin (>7.73 μIU/ml, RR 2.196, 95% CI 1.126-4.292, P=0.021) (Table V).

Similarly, when we performed multivariate analysis, only two factors, the Child-Pugh grade and the level of fasting insulin influenced the prognosis of early stage HCC with a

Table V. Univariate analyses of prognosis factors for HCC of TNM stage I and II.

Variable	Relative risk (95% CI)	P
Onset age, >60 y.o.	1.248 (0.536-2.907)	0.606
Gender, male	1.637 (0.715-3.745)	0.244
BMI, >25.0 kg/m^2	1.488 (0.746-2.967)	0.260
Alcohol intake, ≥80g/day	2.488 (1.160-5.319)	0.019
Background		0.647
non-B, non-C	-	-
HBsAg(+)	1.111 (0.230-5.369)	0.896
HCVAb(+)	1.566 (0.366-6.700)	0.545
Underlying liver diseases and Child-Pugh grade		<0.001
CH	-	-
LC Child-Pugh grade A	2.531 (0.866-7.395)	0.090
LC Child-Pugh grade B	4.582 (1.370-15.323)	0.014
LC Child-Pugh grade C	41.104 (6.403-263.831)	<0.001
Serum ferritin, <185 ng/ml	1.193 (0.621-2.295)	0.596
Serum iron, <141 μg/ml	1.222 (0.641-2.331)	0.542
Fasting insulin, >7.73 μIU/ml	2.196 (1.126-4.292)	0.021
Fasting blood glucose, >110 mg/dl	0.949 (0.118-7.634)	0.961
HOMA-R, >2.0%	4.762 (0.475-47.619)	0.184
Total adiponectin, >6.95 μg/ml	0.921 (0.479-1.767)	0.804
HMW, 3.0 μg/ml	0.799 (0.418-1.529)	0.498
MMW, >1.6 μg/ml	1.171 (0.613-2.232)	0.633
LMW, >2.1 μg/ml	1.038 (0.544-1.984)	0.908
Therapy, TACE or TAI	1.429 (0.743-2.748)	0.285

Table VI. Multivariate analyses of prognosis factors for HCC of TNM stage I and II.

Variable	Relative risk (95% CI)	P
Alcohol intake, ≥80 g/day	2.217 (0.933-5.263)	0.071
Underlying liver diseases and Child-Pugh grade		0.022
CH	-	-
LC Child-Pugh grade A	2.884 (0.975-8.531)	0.056
LC Child-Pugh grade B	3.771 (1.099-12.529)	0.035
LC Child-Pugh grade C	19.039 (2.782-130.298)	0.003
Fasting insulin, >7.73 μIU/ml	2.033 (1.019-4.049)	0.044

significant difference: Child-Pugh grade (grade B, RR 3.771, 95% CI 1.099-12.529, P=0.035, grade C, RR 19.039, 95% CI 2.782-130.298, P=0.003), and the level of fasting insulin (>7.73 μIU/ml, RR 2.033, 95% CI 1.019-4.049, P=0.044) (Table VI).

Association of fasting insulin and total adiponectin level with recurrence-free survival. To evaluate the association of fasting insulin level with the recurrence-free survival time, 59 patients who underwent curative therapy, defined as a condition characterized by the no findings of recurrence over six months after the initial therapy, were extracted from 140 patients and subjected to analysis. Of 59 patients, the mean level of insulin in the low insulin group (<7.73 μIU/ml, n=32) or that in the high insulin group (>7.73 μIU/ml, n=27) was 3.8 μIU/ml or 14.4 μIU/ml, respectively. Fig. 4A indicates the cumulative recurrence-free survival rates of 59 patients who underwent curative therapy. The high insulin group exhibited a lower recurrence-free survival with a significant difference in comparison to the low insulin group (P=0.017).

Similarly, we evaluated the association of the total adiponectin level with the recurrence-free survival time. The mean level of total adiponectin in the low adiponectin group (<6.95 μIU/ml, n=28) or that in the high adiponectin group (≥6.95 μIU/ml, n=31) was 4.5 μIU/ml or 11.7 μIU/ml, respectively. We compared the cumulative recurrence-free survival rates of HCC between the low adiponectin group and the high adiponectin group, but no significant difference was found (Fig. 4B).

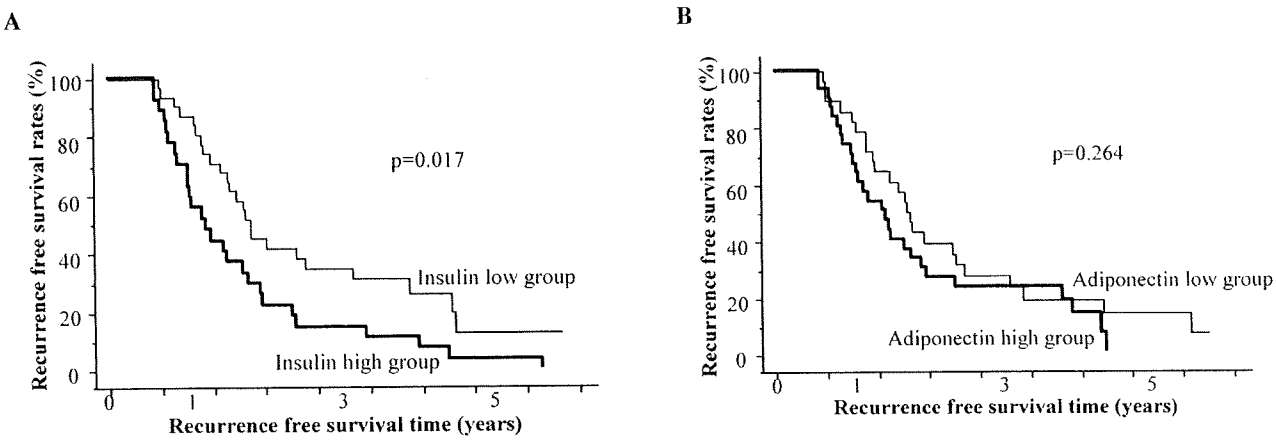

Figure 4. Kaplan-Meier curves for recurrence-free survival in HCC patients who underwent curative therapy (n=59) between low insulin group (thin line) and high insulin group (heavy line) (A) and between low adiponectin group (thin line) and high adiponectin group (heavy line) (B).

Table VII. Univariate analyses of the factor that contribute to recurrence-free survival.

Variable	Relative risk (95% CI)	P
Onset age, >60 y.o.	1.905 (0.951-3.871)	0.069
Gender, male	1.984 (0.355-1.243)	0.201
BMI, >25.0 kg/m^2	2.268 (0.805-4.367)	0.014
Alcohol intake, ≥80 g/day	1.289 (0.653-2.538)	0.465
Background		0.671
Non-B, non-C	-	-
HBsAg(+)	1.302 (0.289-5.855)	0.731
HCVAb(+)	1.566 (0.390-6.773)	0.505
Underlying liver diseases and Child-Pugh grade		0.093
CH	-	-
LC Child-Pugh grade A	2.300 (1.095-4.831)	0.028
LC Child-Pugh grade B	2.883 (1.086-7.650)	0.034
LC Child-Pugh grade C	3.655 (0.774-17.263)	0.102
Serum ferritin, <185 ng/ml	1.157 (0.663-2.019)	0.607
Serum iron, <141 μg/ml	1.379 (0.772-2.464)	0.278
Fasting insulin, >7.73 μIU/ml	1.946 (1.117-3.378)	0.019
Fasting blood glucose, >110 mg/dl	4.975 (0.903-27.778)	0.065
HOMA-R, >2.0%	4.255 (0.816-22.222)	0.086
Total adiponectin, >6.95 μg/ml	1.376 (0.784-2.410)	0.266
HMW, >3.0 μg/ml	1.076 (0.611-1.893)	0.799
MMW, >1.6 μg/ml	1.258 (0.711-2.222)	0.430
LMW, >2.1 μg/ml	1.012 (0.572-1.792)	0.967
Therapy, TACE or TAI	1.165 (0.646-2.101)	0.610

Univariate and multivariate analyses of the factors associated with recurrence-free survival. To clarify the factors that contribute to recurrence-free survival except for tumoral factors, univariate and multivariate analyses were performed using the Cox proportional hazards model in 59 patients who underwent curative therapy. In a univariate analysis, only two

Table VIII. Multivariate analyses of the factors that contribute to recurrence-free survival.

Variable	Relative risk (95% CI)	P
BMI, >25.0 kg/m^2	1.992 (1.026-3.861)	0.042
Fasting insulin, >7.73 μIU/ml	1.767 (1.004-3.117)	0.049

factors significantly influenced the recurrence-free survival: BMI (>25.0 kg/m^2, RR 2.268, 95% CI 0.805-4.367, P=0.014) and the value of insulin (>7.73 μIU/ml, RR 1.946, 95% CI 1.117-3.378, P=0.019) (Table VII). Multivariate analysis showed that both factors influenced the recurrence-free survival with a significant difference: BMI (>25.0 kg/m^2, RR 1.992, 95% CI 1.026-3.861, P=0.042) and the value of insulin (>7.73 μIU/ml, RR 1.767, 95% CI 1.004-3.117, P=0.049) (Table VIII).

Discussion

Several prior studies have reported that the coexistence of DM influences the prognosis of HCC patients (10,11,28,29). However, the mechanism responsible for this finding remains unclear. Since the glucose tolerance of an individual is defined by the potential insulin secretion from ß-cells and by the insulin sensitivity of target tissues including the liver, serum levels of fasting and postprandial insulin could differ in each HCC patient. In addition, advanced liver fibrosis is directly linked to an increase in the insulin resistance in HCV-infected patients (13,30).

In the present study, we therefore focused on the serum level of insulin rather than on the glucose tolerance in the HCC patients. Our study indicates that a high value of fasting insulin heralds not only a poor prognosis in the early stage of HCC but also a high recurrence rate in the curative HCC. There are a few studies on the prognostic value of hyperinsulinemia on patients with HCC. Saito *et al* have demonstrated that the area under the plasma insulin curve for the oral glucose tolerance test can serve as a significant prognostic tool, and can assist in forecasting the doubling time of HCC (16), and that continuous infusion of octreotide in five patients inhibited insulin secretion resulting in a decrease in the HCC growth rate.

Komura *et al* reported that insulin therapy for coexisting DM is an independent risk factor for HCC recurrence after a curative resection (10). Taken together, it is possible that hyperinsulinemia promotes the progression and development of HCC. This is consistent with the results from the following *in vitro* studies, that insulin has the potential to accelerate the growth of hepatoma cells and inhibits apoptosis through the upregulation of Bcl-xl (14), and that insulin stimulates the motility and invasiveness of hepatoma cells (31). In addition, there have been several clinical studies supporting the association between hyperinsulinemia and the advancement of cancers. A high level of fasting insulin is associated with distant recurrence and death in early stage breast cancer (32). High insulin levels are associated with a poorer prognosis in prostate cancer and endometrial cancer, and malignant degeneration of adenomatous polyps (33-36). These findings suggest that, in addition to an effect on glucose metabolism, insulin functions to promote the proliferation and metastasis of various types of cancer cells.

Hyperinsulinemia is inextricably linked to insulin resistance of the peripheral tissues including the liver. In our study, HOMA-R, a good indicator of insulin resistance, was not associated with a poor prognosis in early stage HCC (univariate analysis, P=0.184) and a recurrence-free survival in curative HCC (univariate analysis, P=0.086) although HOMA-R was significantly higher in the high insulin group than in the low insulin group (Table III). It is probably due to the small number of cases used to determine the HOMA-R (28 of 140 subjects or 11 of 59 subjects).

Since adiponectin has a potent insulin-sensitizing effect, we determined its value in HCC patients. In contrast to fasting serum insulin, the mean value of total adiponectin apparently increased with the decline of liver function. The HCC stage did not affect the values of total adiponectin. A similar observation has been reported by Tacke *et al* (23), in which they suggest that the elevation of adiponectin in chronic liver disease is due to the decrease of clearance from the serum, and possibly decreased biliary excretion of adiponectin, and that portal hypertension and the development of HCC do not affect the values of adiponectin. In addition to total adiponectin, we measured the levels of HMW, MMW, and LMW adiponectins. These adiponectins increased in direct relation to the decline in the liver function (data not shown), thus suggesting that higher molecular weight adiponectin is also metabolized by the liver. It is surprising that the values of total, HMW, MMW, and LMW adiponectins showed no significant differences between the high insulin group and the low group (Table III). However, Tacke *et al* have already reported a similar observation that the elevated adiponectin in LC patients is not directly involved in insulin sensitivity. Recently, adiponectin is known to possess antitumoral activity. The circulating adiponectin level is inversely associated with an increased risk of breast cancer, endometrial, prostate, gastric, and colorectal cancer (37-41). Furthermore, Miyazaki *et al* reported that adiponectin shows an antitumor effect against HepG2 hepatoma cells through JNK activation and suppression of STAT3 function (42). However, our study showed that total adiponectin has no impact on the prognosis of any stage of HCC. It is unclear why there is a discrepancy between these literature findings and our own. We are now speculating that certain cirrhotic environments such as advanced liver fibrosis, decreased liver function and portal systemic shunting may diminish the anti-tumoral activity of adiponectin against HCC. Further studies are thus needed to clarify this.

Although the present study is retrospective and involves a limited number of participants, this is a first study indicating that fasting hyperinsulinemia is a risk factor associated with a poor prognosis in the early stage of HCC and a high recurrence rate in the curative HCC. We have to validate our findings with a prospective study and also clarify the mechanism by which insulin impacts the clinical course of HCC. However, our study suggests that treatment modalities which lower the level of fasting insulin could improve the prognosis of the early stage of HCC and reduce the recurrence of HCC.

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Original Article

Hepatitis C virus kinetics during the first phase of pegylated interferon-α-2b with ribavirin therapy in patients with living donor liver transplantation

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Aim: To identify the problems of pegylated interferon (PEG IFN) with ribavirin therapy against hepatitis C virus (HCV) reinfection in living donor liver transplantation (LDLT) patients. HCV kinetics during the PEG IFN with ribavirin therapy were analyzed in LDLT patients, as well as in chronic hepatitis C (CHC) patients.

Methods: The study included 80 consecutive HCV infected patients undergoing PEG IFN with ribavirin therapy (64 CHC and 16 LDLT patients) who attended the Nagasaki University Hospital for an initial visit between January 2005 and December 2007.

Results: The sustained viral response (VR) rate of the CHC group (80%) was superior to the LDLT group (22%). The viral disappearance rate of the CHC group was also superior to the LDLT group, regardless of the HCV serotype. The HCV core antigen (cAg) titer under treatment in the LDLT group was more than that of the CHC group from day 0 to week 12. The HCV cAg decrease rate of the LDLT group on the first day of treatment was less than that of the CHC group.

Conclusion: The HCV infection of a transplanted liver is more refractory to treatment than a non-transplanted liver. The low reduction HCV cAg rate on day 1 is one of the problems of the combination therapy.

Key words: chronic hepatitis C, first phase, hepatitis C virus, interferon, living donor liver transplantation

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is widespread throughout the world. Chronic HCV infection leads to cirrhosis and hepatocellular carcinoma. Liver transplantation for HCV-related liver disease has been an option worldwide.[1] Recently, it has been shown that the prognosis for liver transplanted (LT) patients with HCV-related disease deteriorates over time,[2] thus resulting in a poorer outcome than in the non-HCV course.[3] The transplanted liver for HCV-related disease undergoes a rapidly progressive fibrosis and acute graft failure.[3,4] Consequently, anti-HCV treatment after LT is important for the prognosis. Interferon (IFN) has been recognized as the only treatment method for HCV infection. For the transplanted liver, it is known that IFN treatment improves liver fibrosis or halts the progression.[5] Recently, the combination of pegylated IFN (PEG IFN) with ribavirin was used and produced an excellent result for non-transplanted patients with HCV.[6] However, that was not the case for the HCV re-infected transplanted liver.[7] It is important that the cause of refractory HCV infection in the transplanted liver be more fully clarified. Immunosuppressant therapy, especially with glucocorticoid, has been speculated to be the cause of the refractory nature of the transplanted liver to IFN.[8,9] The cause of this is considered to be that glucocorticoid downregulated the IFN signal transduction in the hepatocytes.[8] The authors recently found that calcineurin inhibitors also inhibited IFN induced STAT-1 phosphorylation and antiviral activity in the HCV

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replicon system.[10] Therefore, the problem of IFN signaling in the hepatocyte induced an IFN refractory condition[11] and decreased the first phase of HCV decline, which was IFN induced HCV decay during the first day of IFN treatment.[12]

In the present study, we attempted to better understand PEG IFN and ribavirin therapy by comparing patients with chronic hepatitis from HCV infection (CHC) with living donor LT (LDLT) patients. When the non-transplanted CHC patients were used as a reference against the HCV reinfected LDLT patients, we expected that the differences in the clinical data in the two groups would help to clarify the problem of IFN refractory HCV infection, and shed light on the analysis of HCV kinetics under IFN and ribavirin treatment, and to elucidate the damaged segment of the IFN induced antiviral mechanism in the LDLT condition.

PATIENTS AND METHODS

Patients

THE PRESENT RESEARCH is a prospective study. The study included 80 consecutive HCV-infected patients undergoing PEG IFN with ribavirin combination therapy (64 CHC and 16 LDLT patients) who attended the Nagasaki University Hospital for an initial visit between January 2005 and December 2007. All patients received the targeted dose of 1.5 μg/kg PEG IFN-α-2b (Pegintron; Schering-Prough K.K., Osaka, Japan) once weekly with daily ribavirin (Rebetol; Schering-Prough K.K., Osaka, Japan) for a total dose of 600 mg (bodyweight < 60 kg), 800 mg (60 kg < bodyweight < 80 kg) or 1000 mg (bodyweight > 80 kg) according to bodyweight (BW). The number of patients who were judged to have obtained a curative effect from IFN therapy was 42 in total, and 12 were LDLT patients. If the HCV-RNA had been negative in the patient serum until 12 weeks after the initiation of treatment or positive at 24 weeks, PEG IFN with ribavirin therapy was stopped at week 48. If the HCV-RNA had been negative from weeks 12 to 24, PEG IFN with ribavirin therapy was continued for 24 weeks to a predetermined 48 weeks. CHC patients were diagnosed on the basis of a persistently raised alanine aminotransferase (ALT) level and biopsy proven disease. All LDLT patients, who had undergone liver transplantation for HCV related cirrhosis at Nagasaki University Hospital from June 2002 to May 2007, had the HCV-RNA in their serum at the commencement of PEG IFN with ribavirin treatment. To prevent HCV related hepatitis after liver transplantation, pre-emptive therapy using IFN is the strategy used at the Nagasaki University Hospital. After the recovery of the general condition without ascites and icterus after transplantation, and establishment of the diagnosis using the liver biopsy, PEG IFN with ribavirin therapy was started. The interval between LDLT and IFN treatment was a mean of 281 days (range 16–989 days). Tacrolimus (Astellas, Tokyo, Japan), an immunosuppressive agent, was used together with steroids for all LDLT patients as the induction therapy. When IFN therapy was commenced, tacrolimus was switched to cyclosporin (Novartis, Tokyo, Japan) in 12/16 cases. A percutaneous liver biopsy assisted by ultrasonography was carried out in all cases. Liver histology was evaluated according to the degree of fibrosis and necroinflammatory activity.[13] The extent of fibrosis (staging) was classified as follows: F1 (periportal expansion), F2 (portoportal septa), F3 (portocentral linkage or bridging fibrosis) and F4 (cirrhosis). The necroinflammatory activity (grading) was classified as follows: A1 (mild), A2 (moderate) and A3 (severe). Liver biopsy specimens were fixed in 10% formalin, embedded in paraffin, cut to a thickness of 4 μm, and subjected to hematoxylin–eosin and Azan–Mallory staining.

Hepatitis C virus kinetics assessment

We compared the HCV viral load in both groups, determined by the HCV core antigen (cAg), at baseline (D0), day 1 (D1), week 1 (W1), week 2 (W2), week 4 (W4), week 8 (W8), week 12 (W12), week 24 (W24) and week 48 (W48). The HCV viral serotype (ST) and HCV cAg were determined using available kits. In this assay, HCV serotypes 1 and 2 correspond to genotypes 1 and 2 of Simmonds' classification,[14] respectively. The HCV cAg correlates with HCV-RNA by quantitative PCR.[15] HCV cAg was measured at the indicated times and HCV-RNA qualitative PCR, the amplicor monitor method, was used after the level was under the detection range of HCV cAg in every month. In the present study, we proposed the calculation of the decreased HCV viral load during PEG IFN with ribavirin treatment and set as follows: a negative HCV cAg was 20 fmol/L and a negative HCV-RNA qualitative PCR was 1 fmol/L.

Clinical and laboratory measurements

The body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Subjects fasted overnight before blood samples were obtained. Venous plasma glucose was measured with an automated analyzer, and basal serum insulin was measured using a standard radioimmunoassay. The index of insulin

resistance and β-cell function was calculated using the fasting value of plasma glucose (we excluded the patients with greater than 130 mg/dL), and the serum insulin level according to the homeostasis model assessment (HOMA) method. HOMA-IR, an insulin resistance marker, is calculated as follows: fasting plasma glucose × fasting insulin/405. HOMA-β, a β-cell function marker, was calculated as follows: 360 × fasting insulin/(fasting plasma glucose-63).[16] White blood cell, red blood cell, platelet, hemoglobin A1c, ALT, aspartate aminotransferase (AST), γ-GTP, total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), free fatty acid (FFA), and ferritin were determined by standard hematometry and laboratory techniques.

Statistical analysis

The data were processed on a personal computer and analyzed using StatView 5.0 (SAS Institute, Cary, NC, USA). Differences between groups were analyzed by Mann–Whitney *U*-test and Pearson χ^2-test. All data in the text and tables are shown as means, unless otherwise indicated. The statistical analysis of the HCV-RNA disappearance rate was by the Kaplan–Meier method with Wilcoxon assay. Values of $P < 0.05$ were considered to be statistically significant.

RESULTS

Differences of patient characteristics

FIRST, THE PRETREATMENT clinical and laboratory characteristics were compared with All-CHC and All-LDLT patients (Table 1). The BW and BMI in the All-CHC group were higher than that of the All-LDLT group. Therefore, the levels of PEG IFN dose per BW and ribavirin dose per BW were even, but the levels of PEG IFN dose and ribavirin dose in the all LDLT group were lower than in the All-CHC group. The HCV viral load in the all LDLT group was greater than that in the All-CHC group and serotype 1 was the majority in the All-LDLT group. In hematology and laboratory data, the red blood cell count and hemoglobin in the All-LDLT group was lower than that of the All-CHC group, and the FFA level was higher in the All-LDLT group. In the histological examination, fibrosis is more advanced in the All-CHC group than in the All-LDLT group. There was the tendency toward higher levels of fasting plasma glucose and lower levels of HOMA-β in the All-LDLT group than in the All-CHC group. Next, we targeted the serotype 1 and a high HCV titer (ST1H group) above 100 KIU/L by the qualitative PCR method or 300 fmol/L of the cAg assay. These were examined in the same way (Table 2). The ST1H group might have shown the same result as the All group, except the levels of fasting plasma glucose and HOMA-β did not differ with ST1H-CHC and ST1H-LDLT. The mean value of fasting plasma glucose (FPG) was higher than the normal range in the LDLT group. The discontinuance rates of treatment were almost equal, 19 cases (29.7%) and 4 cases (25%) in All-CHC and All-LDLT, respectively. The reasons for discontinuance were adverse effects in All-LDLT patients and the refractory nature of viral response in two All-CHC patients.

The HCV infection in the LDLT group is more obstinate than in the CHC group

The response rate and cure rate of PEG IFN with ribavirin therapy were compared with both groups (Table 2A, All group and B, ST1H group). The HCV response rate to treatment, viral response (VR), was determined by the disappearance of HCV-RNA or by the decline of HCV cAg to less than 1/100 before treatment. The cure rate, sustained viral response rate (SVR), was determined by a negative HCV-RNA by qualitative PCR method at 6 months post-termination of treatment. The VR rate at 8 and 12 weeks, but not at 4 weeks, and the PP-SVR in the LDLT group (Table 3A,B) was worse than that in the CHC group. Non-viral responders, who did not achieve HCV-RNA negativity during the treatment, did not show statistical significance in either SG1H group (Table 3B). As a result, we calculated the prediction of the lack of SVR by non-viral response in the LDLT group. The sensitivity, specificity, positive predictive values and negative predictive value were 1, 0, 0.917 and the acalculia for null viral responders at 24 h, 0.7, 1, 1 and 0.25 at 4 weeks, 0.6, 1, 1 and 0.2 at 8 weeks and 0.6, 1, 1 and 0.2 at 12 weeks, respectively.

The disappearance rate of HCV-RNA was evaluated by the Kaplan–Meier method (Fig. 1 ST1H group). The disappearance rate in the LDLT group was statistically lower than the CHC group. Before 14 weeks after the initiation of treatment, the HCV-RNA disappearance case was not apparent in the ST1H group (Fig. 1).

The decline of HCV load, especially early phase, is blocked in the LDLT group

For the analysis of viral kinetics, we evaluated the decline of the HCV load and the decline rate after treatment with particular emphasis of the early phase of treatment, including D1-W12. In the ST1H group (Fig. 2), the decreased rate on D1 in the LDLT group was

Table 1 Difference of characteristics between all chronic hepatitis C cases and all living donor liver transplantation cases

Characteristics	All-CHC ($n = 64$)	All-LDLT ($n = 16$)	*P*-value
Age (years)	58 ± 10.8	58.8 ± 4.62	NS
Sex (male : female)	36:28	7:9	NS
Height (m)	1.60 ± 0.098	1.583 ± 0.010	NS
Bodyweight (kg)	61.0 ± 11.0	54.8 ± 8.52	0.025
Body mass index	23.6 ± 2.94	21.8 ± 2.30	0.022
PEG IFN dose (μg)	80.1 ± 18.7	71.9 ± 33.5	0.035
PEG IFN/BW	1.31 ± 0.304	1.35 ± 0.708	NS
Ribavirin dose (mg)	621.9 ± 151.7	525 ± 100	0.030
Ribavirin/BW	10.2 ± 2.23	9.72 ± 2.04	NS
Serotype (1:2)	45:17	15:1	0.081
HCV cAg (fmol/L)	5773 ± 5609	23144 ± 21059	0.001
WBC (/μL)	5006.3 ± 1335	5918.8 ± 2439	NS
RBC (10^4/μL)	445 ± 41.1	350 ± 56.7	< 0.0001
Hemoglobin (g/dL)	13.8 ± 1.06	10.9 ± 1.85	< 0.0001
Platelet (10^4/μL)	16.4 ± 4.48	18.5 ± 10.6	NS
AST (U/L)	62.9 ± 35	64.3 ± 37.2	NS
ALT (U/L)	85 ± 53.0	89.9 ± 57.1	NS
γ-GTP (U/L)	62.1 ± 56.5	138.9 ± 129.1	0.013
Ferritin (ng/dL)	218 ± 216	254 ± 259	NS
TC (mg/dL)	169.8 ± 26.6	167.3 ± 38.8	NS
TG (mg/dL)	105.3 ± 46.8	122.8 ± 44.8	0.069
HDL (mg/dL)	45.2 ± 11.9	46.6 ± 14.9	NS
LDL (mg/dL)	97.3 ± 24.3	88.8 ± 26.7	NS
FFA (mEq/L)	0.492 ± 0.261	0.686 ± 0.299	0.019
FPG (mg/dL)	91.9 ± 15.4	125.1 ± 56.9	0.090
Insulin (mIU/L)	9.16 ± 5.1	8.34 ± 5.16	NS
HOMA-IR	2.08 ± 1.22	1.75 ± 1.42	NS
HOMA-β	135.4 ± 86.2	89.7 ± 86.9	0.075
Fibrosis	1.86 ± 1.18	0.875 ± 0.806	0.004
Activity	1.03 ± 0.48	1.31 ± 0.48	0.067

Data are shown as the means ± standard deviation and values, with statistical analysis calculated by Mann–Whitney *U*-test for means and Pearson's χ^2-test for values.
Normal values in laboratory tests: ALT (IU/L), 5–40; AST (IU/L), 10–40; γ-GTP (IU/L), < 70 in males, < 30 in females; TC (mg/dL), 150–219; TG (mg/dL), 50–149; FFA (mEq/L), 0.14–0.85; LDL (mg/dL), 70–139; HDL (mg/dL), 40–86 in male, 40–96 in female; hemoglobin (g/dL), 13.5–17.6 in male, 11.3–15.2 in female; WBC (/μL), 3900–9800 in males, 3500–9100 in females; RBC (10^4/μL), 427–570 in males, 376–500 in females; ferritin (mg/dL), 27–320 in males, 3.4–89 in females; platelet (10^4/μL), 13.1–36.2 in males, 13–36.9 in females; insulin (IU/L), 3.06–16.9; FPG (mg/L), 70–109. HOMA-IR, HOMA-β, and BMI are described in the text.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHC, chronic hepatitis C; FFA, free fatty acid; FPG, fasting plasma glucose; HCV cAg, hepatitis C virus core antigen; HDL, high density lipoprotein; HOMA, homeostasis model assessment; LDL, low density lipoprotein; LDLT, living donor liver transplantation; PEG IFN, pegylated interferon; RBC, red blood cell count; TC, total cholesterol; TG, triglyceride; WBC, white blood cell count.

statistically lower than CHC (Fig. 2b) and the viral load of the LDLT group was larger than that in CHC from D0 to W12 (Fig. 2a). The decreased rate at the indicated time without D1 and W12 was not the difference between CHC and LDLT (Fig. 2b). We next analyzed the SG1H-group that matched the pre-treatment HCV cAg titer (Fig. 3). In a similar fashion to Figure 2, the viral load of the matched LDLT group was larger than that of the matched CHC from D1 to W12 (Fig. 3a) and the decreased rate of the matched LDLT group was lower than that of the matched CHC at D1, W2 and W4 (Fig. 3b).

DISCUSSION

IN THE PRESENT prospective study, we compared CHC and LDLT patients treated with PEG IFN and ribavirin for HCV infection. BMI, HCV cAg, red blood

Table 2 Difference of characteristics of serotype 1 and high virus titer between chronic hepatitis C patients and living donor liver transplantation patients

Characteristics	ST1H-CHC ($n = 42$)	ST1H-LDLT ($n = 15$)	*P*-value
Age (years)	58.5 ± 10.8	58.8 ± 4.78	NS
Sex (male : female)	22:20	6:9	NS
Height (m)	1.60 ± 0.10	1.566 ± 0.081	NS
Bodyweight (kg)	61.8 ± 12.1	53.8 ± 7.69	0.02
Body mass index	24.0 ± 2.78	21.9 ± 2.37	0.012
PEG IFN dose (μg)	81.4 ± 19.5	73.3 ± 34.2	0.052
PEG IFN/BW	1.33 ± 0.269	1.39 ± 0.711	NS
Ribavirin dose (mg)	642.8 ± 150.0	520 ± 101.4	0.011
Ribavirin/BW	10.5 ± 2.13	9.80 ± 2.08	NS
HCV cAg (fmol/L)	6969 ± 5281	24674 ± 20856	0.003
WBC (/μL)	5019.0 ± 1294	6033.8 ± 2479	NS
RBC (10^4/μL)	444 ± 40.1	351 ± 58.6	< 0.0001
Hemoglobin (g/dL)	13.9 ± 1.10	10.8 ± 1.88	< 0.0001
Platelet (10^4/μL)	16.7 ± 4.68	18.9 ± 10.8	NS
AST (U/L)	62.1 ± 31.6	64.2 ± 38.5	NS
ALT (U/L)	84.5 ± 51.8	88.0 ± 58.6	NS
γ-GTP (U/L)	64.0 ± 61.7	113.6 ± 83.1	0.036
Ferritin (ng/dL)	206 ± 164.8	204.5 ± 188.4	NS
TC (mg/dL)	172.6 ± 25.7	165.3 ± 39.2	NS
TG (mg/dL)	108.2 ± 52.2	122.9 ± 46.4	NS
HDL (mg/dL)	46.5 ± 11.9	45.4 ± 14.8	NS
LDL (mg/dL)	97.7 ± 25.4	88.6 ± 27.8	NS
FFA (mEq/L)	0.514 ± 0.251	0.693 ± 0.310	0.049
FPG (mg/dL)	92.4 ± 16.4	123.7 ± 58.6	NS
Insulin (mIU/L)	9.06 ± 5.5	8.34 ± 5.16	NS
HOMA-IR	2.07 ± 1.31	1.86 ± 1.38	NS
HOMA-b	128.0 ± 76.2	95.7 ± 86.5	NS
Fibrosis	1.92 ± 1.19	0.933 ± 0.799	0.008
Activity	1.08 ± 0.474	1.33 ± 0.488	0.098

Data are shown as the means ± standard deviation and values, with statistical analysis calculated by Mann–Whitney *U*-test for means and Pearson's χ^2-test for values.
Normal values in laboratory tests are same as in Table 1.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHC, chronic hepatitis C; FFA, free fatty acid; FPG, fasting plasma glucose; HCV cAg, hepatitis C virus core antigen; HDL, high density lipoprotein; HOMA, homeostasis model assessment; LDL, low density lipoprotein; LDLT, living donor liver transplantation; PEG IFN, pegylated interferon; RBC, red blood cell count; TC, total cholesterol; TG, triglyceride; WBC, white blood cell count.

cell, γ-GTP, FFA and liver fibrosis in the pretreatment clinical characteristics were different in both groups (Tables 1,2). The VR rate of the CHC group was superior to that of the LDLT group, and the SVR by per-protocol analysis was also similar in result to the VR (Table 3). The viral disappearance rate of the CHC group was superior to the LDLT group, regardless of the HCV serotype (Fig. 1). The HCV cAg titer under the treatment in the LDLT group was more than that of the CHC group from D0 to W12 (Figs 2a,3a) and the HCV cAg decrease rate of the LDLT group at the D1 was less than that of the CHC group (Figs 2b,3b). We showed that the reinfected HCV to the graft liver was more refractory than the non-transplanted CHC. The PEG IFN and ribavirin dose per BW was an equal dose in both groups. However, it was difficult to determine the pretreatment predictive factors for the LDLT cases, because only one case showed SVR in the LDLT group. Thus, we considered that the difference of the pretreatment clinical characteristics in both groups might be related to the refractory HCV infection.

The pretreated HCV cAg titer is known to be the principal factor for IFN resistance. For CHC and LDLT patients, a high HCV-RNA titer in the pretreatment sera

Table 3 Result of pegylated interferon-α-2b plus ribavirin therapy

A. All cases			
Term	All-CHC	All-LDLT	*P*-value
Viral response 4 weeks	40/60 (67%)	5/12 (42%)	NS
Viral response 8 weeks	47/55 (85%)	6/12 (50%)	0.011
Viral response 12 weeks	43/48 (90%)	6/12 (50%)	0.003
Sustained viral response: ITT	20/42 (45%)	2/12 (20%)	0.054
Sustained viral response: PP	20/28 (80%)	2/9 (22%)	0.008
B. Serotype 1 and high virus titer cases			
Term	ST1H-CHC	ST1H-LDLT	*P*-value
Viral response 4 weeks	24/40 (67%)	5/11 (45%)	NS
Viral response 8 weeks	30/36 (83%)	5/11 (45%)	0.012
Viral response 12 weeks	25/29 (86%)	5/11 (45%)	0.008
Sustained viral response: ITT	8/27 (30%)	1/11 (8%)	NS
Sustained viral response: PP	8/15 (53%)	1/9 (11%)	0.029
Non-virological response: ITT	11/27 (41%)	5/11 (45%)	NS
Non-virological response: PP	4/15 (27%)	4/9 (44%)	NS

Data are shown as relevant numbers/target case numbers (percentage of relevant numbers) with statistical analysis using Pearson's χ^2-test for numbers.
CHC, chronic hepatitis C; ITT, intention to treatment analysis; LDLT, living donor liver transplantation; PP, per-protocol analysis.

is associated with non-responder status for IFN treatment.[7,17] In the LDLT condition, the HCV-RNA titer was rapidly increased after immediately decreasing at transplant and the viral load after several weeks post-LDLT exceeded the value of pre-LDLT.[18] The HCV-RNA titer increased rapidly in patients receiving corticosteroids as part of the immunosuppressant regimen.[18,19] We have speculated that the massive amount of HCV, caused by immunosuppressant therapy after the LDLT, was part of the reason for the IFN refractory status. However, comparisons with the pretreated HCV cAg matched groups (Fig. 3) showed the existence of an important factor other than the pretreatment viral load. It will, therefore, be necessary to analyze this problem by evaluating many factors, for example immunosuppressants[10] and regeneration, in the future.

A high level of γ-GTP was also known to be an important factor for IFN treatment.[7,17] Usually, high levels of γ-GTP and FFA have been linked to insulin resistance.[20,21] Therefore, insulin resistance in the liver is assumed in the condition of IFN resistance. However, the LDLT group had the normal range of HOMA-IR,[16] which was lower than that of the CHC group (Tables 1,2). The HCV infection after liver transplantation is associated with insulin resistance.[22] Immunosuppressants, especially corticosteroids, induced insulin resistance.[23] In the present study, the LDLT group had a disturbance of insulin secretion

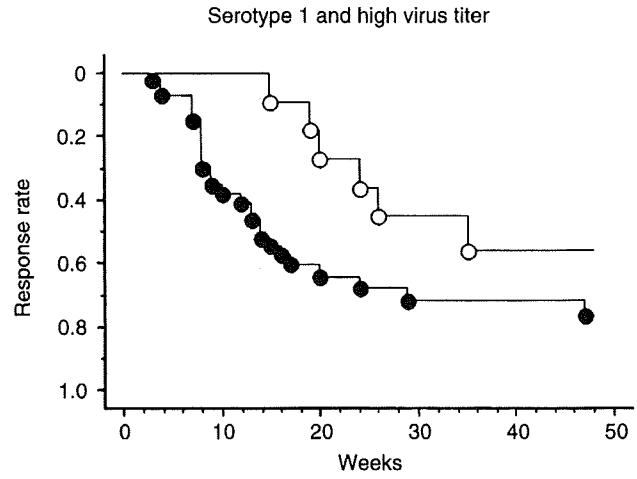

Figure 1 The difference in the hepatitis C ribonucleic acid (HCV-RNA) disappearance rate between the chronic hepatitis C (CHC) group and living donated liver transplantation (LDLT) group during 48 weeks of treatment. HCV-RNA was evaluated by the qualitative PCR method. The disappearance rate was calculated as follows: serum HCV-RNA disappearance case number/all cases in indicated time. The statistical analysis was carried out using the Kaplan–Meier method with the Wilcoxon assay. ST1H group was plotted as the HCV-RNA disappearance line between the white circle of the LDLT group and the black circle of the CHC group. In all cases and the ST1H group, the disappearance rate was statistically significant between the CHC group and the LDLT group ($P < 0.05$).

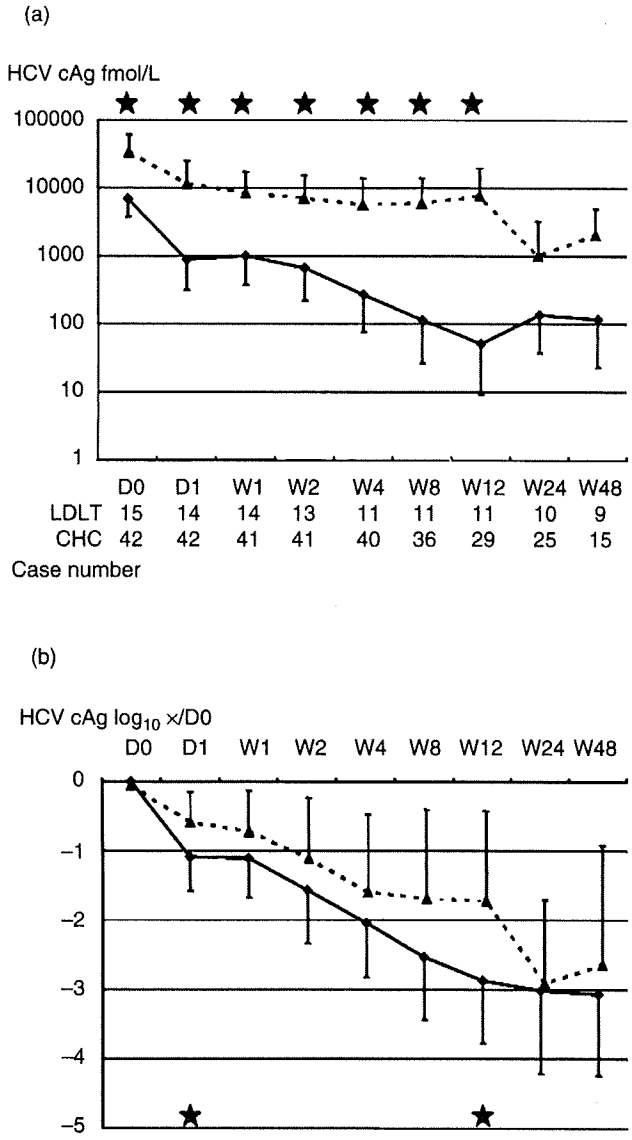

Figure 2 Comparison of viral kinetics between the SG1H-chronic hepatitis C (CHC) group and the SG1H-living donor liver transplantation (LDLT) group during the 48 weeks of treatment. (a) The hepatitis C virus core antigen (HCV cAg) load and (b) reduction rates were plotted by a straight line (SG1H-CHC group), and dotted line (SG1H-LDLT group). The error bar represented the standard deviation. On the *y*-axis, D0 is pretreatment, D1 and WX is time post-treatment day 1 and week X, respectively. The reduction rate was calculated as follows: $\log_{10}$HCV cAg load in indicated time/in D0. HCV cAg titer at the indicated time between SG1H-CHC and SG1H-LDLT were compared. The asterisk mark indicates a significant difference, $P < 0.05$, calculated by Mann–Whitney *U*-test.

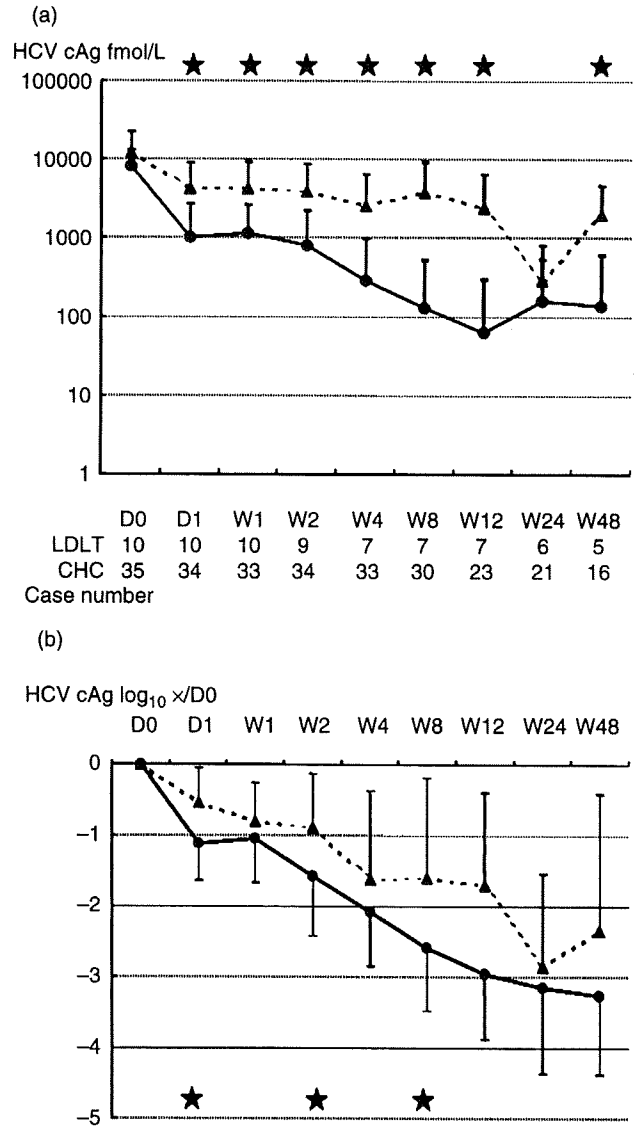

Figure 3 Comparison of viral kinetics between matched pre-treatment hepatitis C virus core antigen (HCV cAg) ST1H-chronic hepatitis C (CHC) group and ST1H- living donor liver transplantation (LDLT) group during 48 weeks of treatment. (a) HCV cAg load and (b) reduction rate were plotted by a straight line (matched SG1H-CHC group) and dotted line (matched SG1H-LDLT group). The error bar represents the standard deviation. The asterisk mark is the significant difference, $P < 0.05$, calculated by Mann–Whitney *U*-test.

rather than insulin resistance and high levels of FPG might be caused by the disturbance of insulin secretion. Therefore, further study is necessary to clarify the relationship between the glucose metabolism and the IFN resistance in LDLT patients. The levels of γ-GTP rise at cholestatic conditions. It was reported that the presence

of a cholestatic profile is associated with an adverse response to IFN treatment in LT.[7] A cholestatic profile provoked the TH2-like lymphocyte response.[19] The authors have previously reported that IL-10, representative of TH2 cytokine, inhibits IFN signaling through an inducible suppressor of cytokine signaling.[24] The high levels of FFA were induced by a catabolic state, such as cirrhosis, and were not fully recovered after LDLT. As a result, the levels of FFA reflected a continuous catabolic state at the beginning of IFN treatment. FFA can induce oxidative stress in various cells,[25,26] and inhibit the IFN induced antiviral gene induction through the inactivation of Jak-1 and Tyk-2.[27] Therefore, we are speculating that high levels of γ-GTP and FFA in the LDLT group have the ability to inhibit IFN signaling as much as in the CHC patients.

We are paying attention to the viral decline of D1/D0 (Figs 2b,3b). The decreased rate of D1 is named as the first phase of HCV decline and is the predictor of SVR.[28,29] The first phase influenced the second phase, which is the decline of HCV after D2.[28] The IFN induced antiviral gene products were considered to be very important for antiviral activity.[11] The expressions of the IFN stimulating genes (ISG) were associated with the early phase of the decline[11] and it was reported that the lack of ISG caused early liver fibrosis in the LT patients with HCV.[30] In the LDLT group, the reduced HCV cAg decreased the rate of D1 and this might be part of the cause of being refractory to IFN. We speculate that an IFN signaling disturbance, related to high levels of γ-GTP and FFA, might have triggered the adverse effect to the HCV cAg decreased rate of D1.

In summary, it became clear that the viral response and SVR is worse in the LDLT group. The first phase of viral decay, the decreased rate of D1/D0, also declined in the LDLT group. High levels of γ-GTP and FFA in the pretreatment sera might also be related to IFN-signaling damage in hepatocytes. At the initiation of pre-emptive therapy, HCV had also been increasing in the graft liver and the catabolic status of energy did not recover for the relatively small size of the graft liver. When beginning treatment for an HCV infection after LT, we should carefully take into account the timing of IFN initiation, in addition to the types of immunosuppressants used.

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